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The multifaceted roles of Slits and Robos in cortical circuits: from proliferation to axon guidance and neurological diseases

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Slit repulsion, mediated by Robo receptors, is known to play a major role in axon guidance in the nervous system. However, recent studies have revealed that in the mammalian cortex these molecules are highly versatile and that their function extends far beyond axon guidance. They act at all phases of development to control neurogenesis, neuronal migration, axon patterning, dendritic outgrowth and spinogenesis. The expression of Robo receptors in cortical and thalamocortical axons (TCAs) is tightly regulated by a combination of transcription factors (TFs), proteases and activity. These findings also suggest that Slit and Robos have influenced the evolution of cortical circuits. Last, novel genetic evidence associates various neurological disorders, such as autism, to abnormal Slit/Robo signaling.

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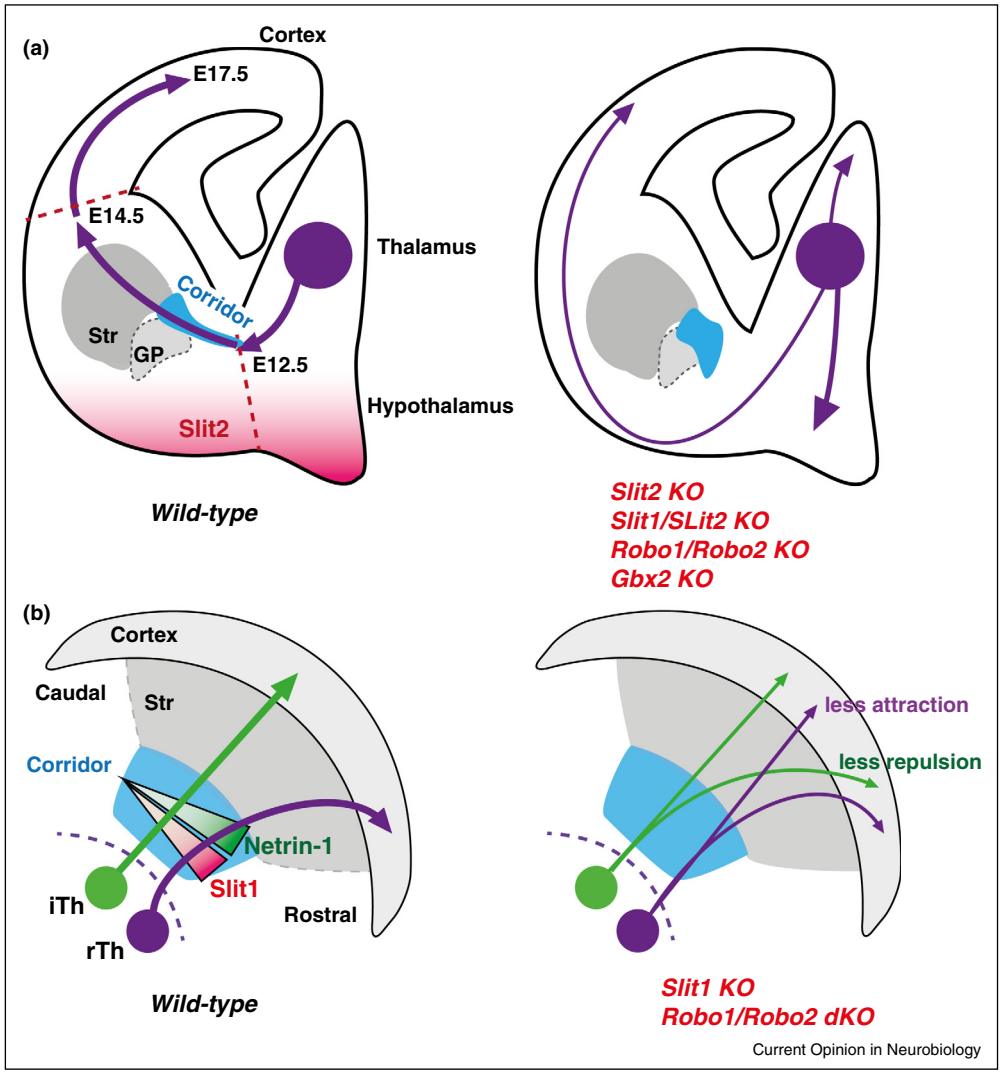
Introduction

Slits are secreted ligands of the roundabout (Robo) receptors. They control commissural axon guidance in the central nervous system (CNS) and a wide range of cell–cell interactions outside the CNS [1]. In mammals, mounting evidence suggests that Slits and Robo receptors are primordial for the development of cortical circuits. In addition, mutations in genes of the Slit–Robo families have been described in a variety of neurological and neuropsychiatric disorders [2]. Here, we will discuss recent findings that shed light on functions of these molecules in the cortex. Given that cortical networks imbalances are thought to be the underlying cause of most neuropsychiatric diseases, we will illustrate Slit–Robo function in various aspects of cortical development and plasticity.

Slit/Robo signaling controls the development of thalamocortical projections

In mammals, there is a mandatory relay for virtually all incoming sensory inputs which are then dispatched to their appropriate cortical areas. The thalamus receives reciprocal projections from the cortex and the two types of axons guide each other during development [3]. In the mouse, thalamocortical axons (TCAs) start extending from the diencephalon into the ventral telencephalon around E12.5, cross the striatum and reach the base of the cortex around E14.5. Their growth across the striatum requires a corridor of guidepost neurons (also called corridor cells), which originate in the lateral ganglionic eminence (LGE) [4] (Figure 1a). Corridor cells express guidance cues such as neuregulins, which promote the extension of TCAs [4]. The topographical organization of TCAs that will be translated to the cortex is also pre-patterned within the corridor. Recent studies have shown that Slit/Robo signaling, directly or indirectly, plays a pivotal role in the development of thalamocortical projections, acting at all choice points along their pathway (Figure 1). Previous studies evidenced that Slit2 from the hypothalamus and diencephalic midline repel TCAs toward the ventral telencephalon [5]. In knockout mice (KO) lacking Slit2 or Robo1 and Robo2, a subset of TCAs invades the hypothalamus and fails to enter the telencephalon [5]. Two groups have shown that the LIM homeodomain 2 (Lhx2) and gastrulation brain homeobox gene 2 (Gbx2) transcription factors (TFs) regulate the expression of Robo receptors in TCAs [6,7]. Both TFs are expressed by postmitotic thalamic neurons during axonogenesis and in *Gbx2* KO embryos most TCAs are unable to enter the telencephalon [6]. Early TCA pathfinding defects (such as ectopic hypothalamic projections) were also observed following targeted deletion of Gbx2, or Lhx2 overexpression in thalamic neurons, supporting a cell-autonomous function of these TFs in axon guidance [7]. Most newly born thalamic neurons express Robo2 at similar levels but the expression of Robo1 is higher in the caudal thalamus where Lhx2 expression is lowest. In *Gbx2* and *Lhx2* mutants, the expression pattern of Robo1 and Robo2 on TCAs was altered. The conditional inactivation of Lhx2 in the thalamus induces a significant upregulation of *Robo1*/*Robo2* transcripts, whereas Lhx2 overexpression had an opposite effect [7]. The presence of specific binding sites in regulatory elements of *Robo* genes suggests that Lhx2 directly represses their transcription. By contrast, Gbx2 appears to activate Robo2 expression indirectly, by inhibiting the transcription of LMO3 (LIM-domain-only 3) a negative regulator of LIM TFs.

Figure 1



Role of Slits and Robos in the development of thalamocortical axons. **(a)** In wild-type mice, Slits are highly expressed in ventral telencephalon and diencephalon, repelling TCAs (purple) away from these domains. Slit repulsion also acts on the positioning corridor cells which constitute a permissive bridge for TCAs in the ventral telencephalon. When Slit/Robo expression is altered, some TCAs are misrouted to the dorsal and ventral diencephalon. Corridor cell migration is also perturbed preventing many TCAs from growing across the striatum. **(b)** Two parallel expression gradients of Slit1 and Netrin-1 within the corridor control the sorting of TCAs along the cortex rostro-caudal axis. See text for details. *Abbreviations*: E, embryonic day; GP, globus pallidus; iTh, intermediate thalamus; Str, striatum; rTh, rostral thalamus.

Although it is still unclear if Lhx2 always positively [6] or negatively [7] controls Robo2 expression in TCAs, there is a consensus in favor of a tight regulation of Robo1/Robo2 expression level in TCAs by Lhx2 and Gbx2 TFs and its importance for TCAs guidance. The abnormal combination of Robo1 and Robo2 receptors on TCAs from *Gbx2* and *Lhx2* KO might also perturb their adhesive properties and fasciculation.

Another elegant study [8•] showed that Slit/Robo repulsion controls the migration of corridor cells from the LGE. In *Slit2* and *Robo1/Robo2* KOs TCAs cannot enter the corridor, which is distorted and points toward the midline instead of the cortex (Figure 1a). *In vitro* transplantation of a wild-type corridor allows TCAs to reach the cortex arguing against a direct role of Robo in TCAs at this level. Another study revealed that Robo expression in TCAs controls their sorting in the ventral telencephalon [9] (Figure 1b). Slit1 is differentially expressed in the corridor in a high-rostral to low-caudal gradient. TCAs express Robo receptors and are repelled by Slits in 3D-gel cultures. In *Slit1* KO, TCAs from the intermediate thalamus invade a cortical domain normally devoted to rostral TCAs suggesting that they are normally repelled from it by Slit1. Surprisingly, in *Slit1* KO rostral TCAs invade caudal cortical regions suggesting that in normal

condition they are restrained from leaving the rostral domain despite the high level of Slit1. A combination of 3D-gel cultures, turning assay and axonal tracing solved this paradox. The chemoattractant Netrin-1 is expressed in the corridor in a gradient similar to Slit1 and TCAs express several of its receptors. Although Netrin-1 alone is unable to attract rostral TCAs, it does so when combined with Slit to which rostral TCAs become unresponsive [9]. A recent study [47] showed that this differential response to netrin involves the FLRT3 receptor. This was unexpected as in spinal cord commissural axons, Slit silences Netrin-1 attraction rather than the opposite [10].

The last phase of TCAs extension is also controlled by Robo1 [11••]. Time-lapse analysis showed that TCAs significantly decelerate upon reaching the cortex. This involves intrinsic molecular changes as the velocity of axons from dissociated thalamic neurons decreases with the age of the donor embryo. Interestingly, there is a parallel decrease of the spontaneous activity of thalamic neurons (lower frequency of calcium transients and resting membrane potential) when their axons enter the cortex suggesting that neuronal activity acts as an accelerator of TCA extension. Once more, Robo1 was identified as one of the gene whose transcription is inhibited by activity in thalamic explants. TFs known to be regulated by calcium, such as *NF-κB*, appear to be involved. Together these data indicate that Robo1 sets the sensitivity of TCAs to Slit, acting as a molecular brake in TCA axons [11••]. This model is further supported by a premature cortical invasion by TCAs in Robo1 and Slit1 knockouts. However, TCAs from *Gbx2* and *Lhx2* KO express higher levels of Robo1 but yet enter the cortex prematurely [6,7].

Slit/Robo and the development of callosal projections

Slit/Robo function in the development of the major forebrain commissure, the corpus callosum (CC), has been extensively studied. In *Robo1/2* and *Slit2* mutants, callosal axons aberrantly project ventrally into the septum and hippocampal and callosal commissural axons are intermingled [12,13]. A subset of axons still cross the midline in *Robo1/2* double KOs, but *Slit1/2* double mutants are almost acallosal. A recent study, using dMRI (diffusion magnetic resonance imaging) showed that Slit2 still acts on callosal axons after midline crossing [14] and that in *Slit2* KO, callosal axons do not project properly to their respective homotopic regions in the contralateral cortex. However, *Robo1* KO do not show defects suggesting that Robo1 is not the sole receptor mediating Slit responses in this system. A novel putative Slit receptor, EVA1C (enhancer of ventral-axon guidance defects of unc40 mutants), initially identified in *C. elegans*, is expressed in developing callosal axons and may complete the picture of Slit-mediated guidance herein [15]. The bHLH TFs *Neurod2/6* control the fasciculation of callosal axons and Robo1 is upregulated in the cortex of Neurod2/6 double mutants [16]. However, since Neurod2/6 act as transcriptional enhancers, transcriptional regulation of Robo1 is likely regulated by a different mechanism.

Other studies suggest that Slit1/3 act indirectly on corticocortical projections by acting on the migration of midline glial populations which pattern this tract [14]. The correct positioning of Slit2 expressing midline glial structures also requires the heparan sulfotransferase Hs6st1, a key modifying enzyme in the biosynthetic pathway of heparan sulfate proteoglycans [17]. Last, Slit2 expression in glia is under the transcriptional control of Gli3. In absence of Gli3, Slit2 expression in midline glia expands toward the septum and perturbs corpus callosum development [18].

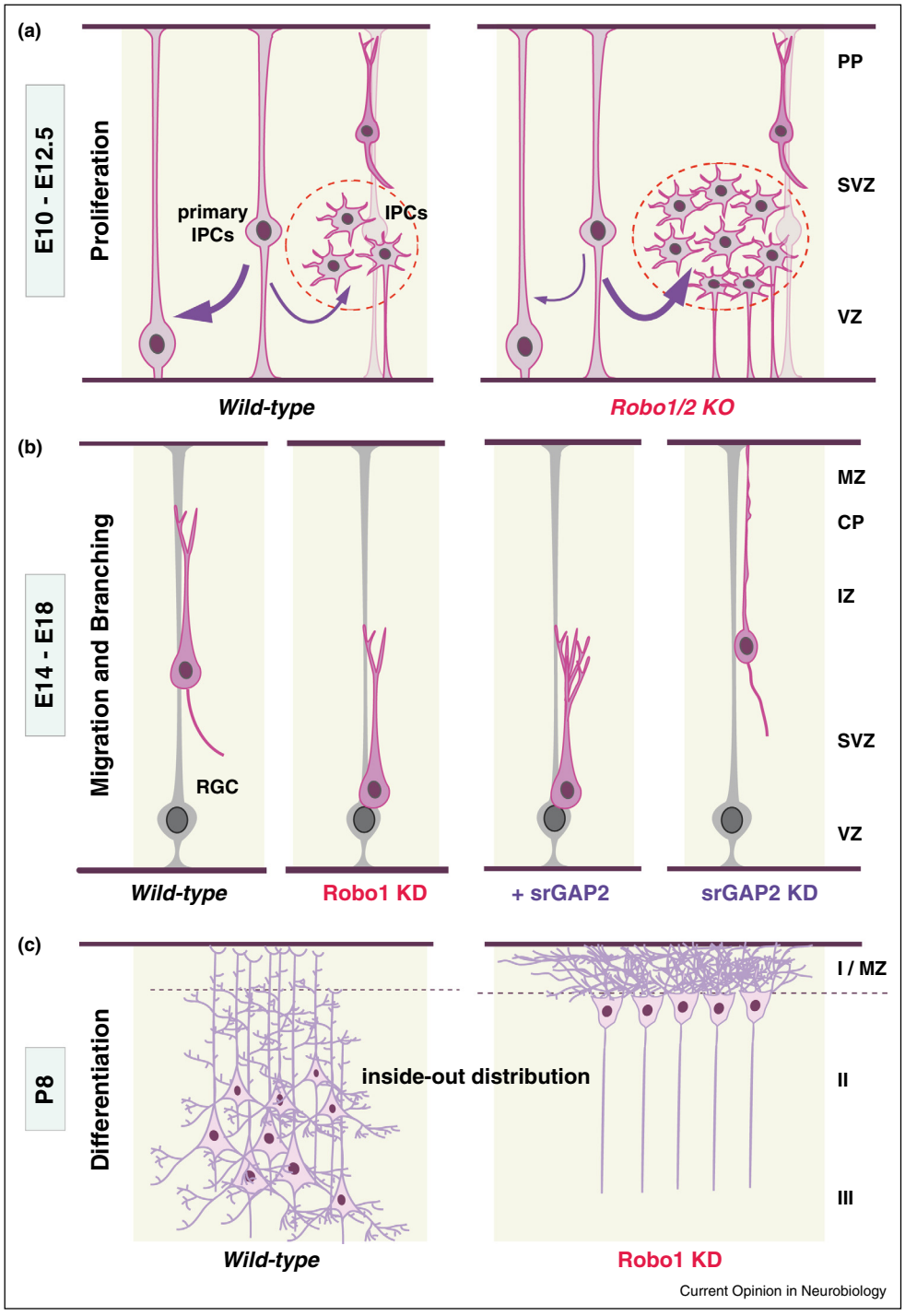
New roles for Slits and Robos in the migration of cortical neurons

The six layers of the mammalian neocortex contain two main types of neurons, interneurons and pyramidal neurons, which originate from distinct progenitors, outside and within the dorsal telencephalon respectively.

Cortical interneurons (CINs) arise primarily from the medial ganglionic eminence (MGE), and migrate tangentially to the cortex. Their migration is escorted by interplay of repulsive cues (such as secreted semaphorins) from the striatum [19] and concerted attraction mediated by the cortex [20]. CINs migrate normally across the ventral pallium and cortex in *Slit1/2* KOs [20]. However, in *Robo1* KO, some interneurons abnormally invade the striatum [21•] suggesting that Robo1 can function independently of Slits [22]. Interestingly, a similar phenotype was described in mice deficient for the class 3 Semaphorin receptor Neuropilin1 (Nrp1) [19]. It was also found that Robo1 can form a complex with Nrp1 and Nrp2 and that in *Robo1* KO, the expression of Nrp1 and its coreceptors PlexinA1/A2 is downregulated in MGE neurons. This could explain why $Robo1^{-/-}$ MGE neurons are less responsive to Sema3A/F and penetrate the striatum where these repulsive cues are expressed [22]. The exact regulatory mechanism is unknown, but in cancer cells [23] Robo1 proteolytic cleavage by metalloproteases and gamma secretases releases an intracellular fragment, which translocates to the nucleus and could regulate the transcription of downstream genes. This renders it possible that neuropilins and plexins may be transcriptional targets of Robo1.

Slit–Robo signaling also regulates the positioning of radially migrating pyramidal neurons within cortical layers. Knockdown of Robo1 *in vivo* leads to a migration delay of cortical pyramidal neurons entering the cortical plate and their distribution in layer II/III is altered (Figure 2). This seems to reflect a specific requirement for Robo1 in the positioning of these neurons rather than a

Figure 2



Function of Slit and Robo in the developing cortex. **(a)** Intermediate cortical progenitors (IPCs) are born in the ventricular zone (VZ) on day E10–E12.5 and proliferate in the subventricular zone (SVZ). They then detach from the apical ventricular surface and migrate toward the preplate region (PP) under the pial surface. In *Robo1* KO, the pool of IPCs is increased and the speed of mitosis is decreased. The rate of neurogenesis, however, is not altered, possibly due to increased adhesion at the ventricular surface. **(b)** As cortical expansion through neurogenesis and migration progresses, prospective pyramidal projection neurons migrate along radial glia cells (RGCs) toward the marginal zone. Migratory speed is reduced in Robo1-deficient neurons and in neurons overexpressing srGAP2 which also show increased neurite branching. Conversely, srGAP2 knockdown decreases neurite branching and hence accelerates migration. **(c)** Consequence of Robo1 knockdown on cortical development. Wildtype layer II/III pyramidal neurons are distributed homogenously throughout their homing layers with their apical dendrites oriented toward the cortical surface. By contrast, the cell bodies of Robo1-deficient neurons remain close to the pial surface. Apical dendrite branching is significantly increased in the absence of Robo1 and the characteristic inside-out distribution of projection neurons and possibly their polarity are altered.

proliferative defect [24]. When electroporated with an shRNA against Robo1, pyramidal neurons in layer II/III form a compact cell-dense rim right beneath the marginal zone, owing to a failure of these cells to descend into deeper cortical layers during postnatal development. Furthermore, after Robo1 loss-of-function, the partition of early and late born neurons in an inside-out manner was lost in layer II/III neurons (Figure 2).

Slit function in the development of dendrites and spines in cortical neurons

In mammals, Slit2 was purified as a factor promoting axonal branching of sensory neurons and this role has since been validated by multiple *in vivo* studies [25,26]. Slit/Robo signaling also controls dendritic development, such as in the cerebellum where Slit2 and Robo2 act in a cell autonomous manner in Purkinje cells, mediating self-avoidance between dendritic branches [27]. In the developing cortex, it was previously shown that Slits promote the extension and branching of pyramidal cell dendrites [28]. A novel study has confirmed these results [29] and showed that this activity involves NCK2, a mammalian ortholog of Dock, an adaptor protein which in Drosophila links Robo receptors to the cytoskeleton [30]. In addition, Robo1 knockdown perturbs the development of pyramidal neuron dendrites [24] (Figure 2).

Novel data suggest that signaling downstream of Slit/Robo influences the growth of the dendritic spines on cortical neurons. Slit–Robo GTPase activating proteins (srGAP1,2,3) are regulators of the actin cytoskeleton through their inhibitory action on small Rho GTPases (Rac1, RhoA, Cdc42). SrGAPS were identified in a two-hybrid screen as downstream components of the Slit/Robo pathway in forebrain neuroblasts [31]. Their function has since been investigated in a variety of contexts, surprisingly without a global emphasis on the requirement of interaction with a Robo receptor.

Through its N-terminal F-BAR domain, srGAP2 induces filopodial membrane deformations. This increases neurite branching in cortical neurons but reduces migration speed [32] (Figure 2). Furthermore, while the F-BAR domain of srGAP1 attenuates the formation of cellular protrusions in cortical neurons, srGAP2 and srGAP3 promote it [33]. Quite remarkably, a segmental gene duplication of the srGAP2 gene in humans leads to the expression of two srGAPs with a truncated F-BAR domain (srGAP2B, srGAP2C), which inhibit the action of srGAP2A, their ancestral counterpart [34••,35]. SrGAP2A promotes dendritic spine maturation and limit their density whereas srGAP2C boosts spine density at the expense of delayed maturation. This human-specific trait leads to increased spine density providing an evolutionary explanation for different morphological features in cortical structures in humans and rodents. How this is linked to Slit–Robo signaling remains obscure and it would be worth investigating the function of srGAPs in a Robo-deficient background. This might also open some therapeutic perspectives as srGAP3 (also known as WRP or MEGAP) plays a role in mental retardation [36], long-term memory and learning [37•].

Proliferation of cortical progenitors

Robo1/2 are expressed in neuronal progenitors and intriguing new studies suggest that they control neuronal proliferation. A higher proliferation of CIN progenitors had been observed in the MGE of *Robo1* KO and *Slit1/2* double KO [38]. However, in the mature cortex, the number of interneurons was only increased in *Slit1/2* KO.

A recent analysis of neuronal proliferation in *Robo1/2* double KO detected a reduction in mitoses across a wide range of proliferative zones within the CNS from the spinal cord to the cortex, which is thinner in mutants [39•]. In the cortex, the absence of Robo1/2 receptors perturbs the renewal of primary progenitors in the ventricular zone (VZ) and generates a larger pool of intermediate progenitor precursor cells (IPCs) in the subventricular zone (SVZ; Figure 2). The proliferation rate of IPCs is also significantly reduced. A similar phenotype was observed in *Slit1/2* double KO. Furthermore, in *Robo1/2* KO a higher proportion of IPCs is connected to the apical surface, suggesting that this perturbs their exit from the VZ and thereby their ability to divide in the SVZ. Therefore, Slits diffusing from the ventricle could stimulate the detachment of IPCs from the ventricular surface through Robo receptors. A candidate gene approach among known mediators of neuronal proliferation revealed that Robo1/2 might control neurogenesis by positively regulating the expression of the Notch effector Hes1 (a basic-loop-helix TF) in cortical progenitors. In Neuro2a cells, Robo1 intracellular domain is required to activate the transcription of Hes1. As Robo1 can be cleaved intracellularly and enter the nucleus [23], these data provide a possible framework for transcriptional regulation of target genes by Robo receptors.

Conclusions

The findings summarized herein illustrate that our understanding of Slit–Robo signaling has already tremendously expanded beyond the classical role in midline axon guidance. Given that Slits and Robos are continuously expressed during embryogenesis and adulthood [40,41], it is tempting to speculate that these proteins are involved in the remodeling and plasticity of neural circuits. It will be worth exploring the clinical potential of these proteins in neuropsychiatric disorders, given that preliminary data already point to a possible function in neurodevelopmental disorders such as dyslexia or autism [42,43]. *ROBO* genes could represent biomarkers in autistic patients [44,45]. Moreover, Slit/Robo function is perturbed in presenilin-1 KO [46•], suggesting that abnormal Slit signaling might contribute to neurodegenerative

diseases such as Alzheimer. A recent study [47] showed that FLRT3 forms a complex with Robo1 which controls Netrin-1 attraction in TCAs.

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Ypsilanti AR, Zagar Y, Chedotal A: **Moving away from the midline: new developments for Slit and Robo**. *Development* 2010, **137**:1939-1952.

2. Lamminmaki S, Massinen S, Nopola-Hemmi J, Kere J, Hari R: **Human ROBO1 regulates interaural interaction in auditory pathways**. *J Neurosci* 2012, **32**:966-971.

3. Deck M, Lokmane L, Chauvet S, Mailhes C, Keita M, Niquille M, Yoshida M, Yoshida Y, Lebrand C, Mann F *et al.*: **Pathfinding of corticothalamic axons relies on a rendezvous with thalamic projections**. *Neuron* 2013, **77**:472-484.

4. Lopez-Bendito G, Cautinat A, Sanchez JA, Bielle F, Flames N, Garratt AN, Talmage DA, Role LW, Charnay P, Marin O *et al.*: **Tangential neuronal migration controls axon guidance: a role for neuregulin-1 in thalamocortical axon navigation**. *Cell* 2006, **125**:127-142.

5. Bagri A, Marin O, Plump AS, Mak J, Pleasure SJ, Rubenstein JL, Tessier-Lavigne M: **Slit proteins prevent midline crossing and determine the dorsoventral position of major axonal pathways in the mammalian forebrain**. *Neuron* 2002, **33**:233-248.

6. Chatterjee M, Li K, Chen L, Maisano X, Guo Q, Gan L, Li JY: **Gbx2 regulates thalamocortical axon guidance by modifying the LIM and Robo codes**. *Development* 2012, **139**:4633-4643.

7. Marcos-Mondejar P, Peregrin S, Li JY, Carlsson L, Tole S, Lopez-Bendito G: **The lhx2 transcription factor controls thalamocortical axonal guidance by specific regulation of robo1 and robo2 receptors**. *J Neurosci* 2012, **32**:4372-4385.

8. • Bielle F, Marcos-Mondejar P, Keita M, Mailhes C, Verney C, Nguyen Ba-Charvet K, Tessier-Lavigne M, Lopez-Bendito G, Garel S: **Slit2 activity in the migration of guidepost neurons shapes thalamic projections during development and evolution**. *Neuron* 2011, **69**:1085-1098.

This elegant study shows that a change in the expression pattern of Slit2 in the subpallium of mammals compared to birds might have deviated inward the migration of a population of LGE neurons which promotes the growth of thalamocortical axons. This could have facilitated the development of reciprocal connections between the thalamus and the cortex.

9. Bielle F, Marcos-Mondejar P, Leyva-Diaz E, Lokmane L, Mire E, Mailhes C, Keita M, Garcia N, Tessier-Lavigne M, Garel S *et al.*: **Emergent growth cone responses to combinations of Slit1 and Netrin 1 in thalamocortical axon topography**. *Curr Biol* 2011, **21**:1748-1755.

10. Stein E, Tessier-Lavigne M: **Hierarchical organization of guidance receptors: silencing of netrin attraction by slit through a Robo/DCC receptor complex**. *Science* 2001, **291**:1928-1938.

11. •• Mire E, Mezzera C, Leyva-Diaz E, Paternain AV, Squarzoni P, Bluy L, Castillo-Paterna M, Lopez MJ, Peregrin S, Tessier-Lavigne M *et al.*: **Spontaneous activity regulates Robo1 transcription to mediate a switch in thalamocortical axon growth**. *Nat Neurosci* 2012, **15**:1134-1143.

By combining electrophysiology, time-lapse recording and mouse genetics the authors provide compelling evidence supporting a modulation of Robo expression by activity in growing thalamocortical axons. They also propose that this mechanism sets the speed of axonal elongation *in vivo*.

12. Lopez-Bendito G, Flames N, Ma L, Fouquet C, Di Meglio T, Chedotal A, Tessier-Lavigne M, Marin O: **Robo1 and Robo2 cooperate to control the guidance of major axonal tracts in the mammalian forebrain**. *J Neurosci* 2007, **27**:3395-3407.

13. Andrews W, Liapi A, Plachez C, Camurri L, Zhang J, Mori S, Murakami F, Parnavelas JG, Sundaresan V, Richards LJ: **Robo1 regulates the development of major axon tracts and interneuron migration in the forebrain**. *Development* 2006, **133**:2243-2252.

14. Unni DK, Piper M, Moldrich RX, Gobius I, Liu S, Fothergill T, Donahoo AL, Baisden JM, Cooper HM, Richards LJ: **Multiple Slits regulate the development of midline glial populations and the corpus callosum**. *Dev Biol* 2012, **365**:36-49.

15. James G, Foster SR, Key B, Beverdam A: **The expression pattern of EVA1C, a novel Slit receptor, is consistent with an axon guidance role in the mouse nervous system**. *PLoS ONE* 2013, **8**:e74115.

16. Bormuth I, Yan K, Yonemasu T, Gummert M, Zhang M, Wichert S, Grishina O, Pieper A, Zhang W, Goebbels S *et al.*: **Neuronal basic helix-loop-helix proteins Neurod2/6 regulate cortical commissure formation before midline interactions**. *J Neurosci* 2013, **33**:641-651.

17. Conway CD, Howe KM, Nettleton NK, Price DJ, Mason JO, Pratt T: **Heparan sulfate sugar modifications mediate the functions of slits and other factors needed for mouse forebrain commissure development**. *J Neurosci* 2011, **31**:1955-1970.

18. Amaniti EM, Hasenpusch-Theil K, Li Z, Magnani D, Kessaris N, Mason JO, Theil T: **Gli3 is required in Emx1+ progenitors for the development of the corpus callosum**. *Dev Biol* 2013, **376**:113-124.

19. Marin O, Yaron A, Bagri A, Tessier-Lavigne M, Rubenstein JL: **Sorting of striatal and cortical interneurons regulated by semaphorin–neuropilin interactions**. *Science* 2001, **293**:872-875.

20. Marin O, Plump AS, Flames N, Sanchez-Camacho C, Tessier-Lavigne M, Rubenstein JL: **Directional guidance of interneuron migration to the cerebral cortex relies on subcortical Slit1/2-independent repulsion and cortical attraction**. *Development* 2003, **130**:1889-1901.

21. • Barber M, Di Meglio T, Andrews WD, Hernandez-Miranda LR, Murakami F, Chedotal A, Parnavelas JG: **The role of Robo3 in the development of cortical interneurons**. *Cereb Cortex* 2009, **19**:i22-i31.

This work provides the first evidence of a direct link between Robo and semaphorin signaling pathways in neuronal migration. Robo1 is shown to regulate the expression of neuropilin-1 and neuropilin-2, two receptors for class 3 semaphorins, on cortical interneurons. This mechanism prevents interneurons from entering the striatum.

22. Hernandez-Miranda LR, Cariboni A, Faux C, Ruhrberg C, Cho JH, Cloutier JF, Eickholt BJ, Parnavelas JG, Andrews WD: **Robo1 regulates semaphorin signaling to guide the migration of cortical interneurons through the ventral forebrain**. *J Neurosci* 2011, **31**:6174-6187.

23. Khusial PR, Vadla B, Krishnan H, Ramlall TF, Shen Y, Ichikawa H, Geng JG, Goldberg GS: **Src activates Abl to augment Robo1 expression in order to promote tumor cell migration**. *Oncotarget* 2010, **1**:198-209.

24. Gonda Y, Andrews WD, Tabata H, Namba T, Parnavelas JG, Nakajima K, Kohsaka S, Hanashima C, Uchino S: **Robo1 regulates the migration and laminar distribution of upper-layer pyramidal neurons of the cerebral cortex**. *Cereb Cortex* 2013, **23**:1495-1508.

25. Wang KH, Brose K, Arnott D, Kidd T, Goodman CS, Henzel W, Tessier-Lavigne M: **Biochemical purification of a mammalian slit protein as a positive regulator of sensory axon elongation and branching**. *Cell* 1999, **96**:771-784.

26. Ma L, Tessier-Lavigne M: **Dual branch-promoting and branch-repelling actions of Slit/Robo signaling on peripheral and central branches of developing sensory axons**. *J Neurosci* 2007, **27**:6843-6851.

27. Gibson DA, Tymanskyj S, Yuan RC, Leung HC, Lefebvre JL, Sanes JR, Chédotal A, Ma L: **Dendrite self-avoidance requires cell-autonomous Slit/Robo signaling in cerebellar Purkinje cells**. *Neuron* 2014, **81**:1040-1056 http://dx.doi.org/10.1016/j.neuron.2014.01.009.

28. Whitford KL, Marillat V, Stein E, Goodman CS, Tessier-Lavigne M, Chedotal A, Ghosh A: **Regulation of cortical dendrite development by Slit–Robo interactions**. *Neuron* 2002, **33**:47-61.

29. Round JE, Sun H: **The adaptor protein Nck2 mediates Slit1-induced changes in cortical neuron morphology**. *Mol Cell Neurosci* 2011, **47**:265-273.

30. Fan X, Labrador JP, Hing H, Bashaw GJ: **Slit stimulation recruits Dock and Pak to the roundabout receptor and increases Rac activity to regulate axon repulsion at the CNS midline**. *Neuron* 2003, **40**:113-127.

31. Wong K, Ren XR, Huang YZ, Xie Y, Liu G, Saito H, Tang H, Wen L, Brady-Kalnay SM, Mei L *et al.*: **Signal transduction in neuronal migration: roles of GTPase activating proteins and the small GTPase Cdc42 in the Slit–Robo pathway**. *Cell* 2001, **107**:209-221.

32. Guerrier S, Coutinho-Budd J, Sassa T, Gresset A, Jordan NV, Chen K, Jin WL, Frost A, Polleux F: **The F-BAR domain of srGAP2 induces membrane protrusions required for neuronal migration and morphogenesis**. *Cell* 2009, **138**:990-1004.

33. Coutinho-Budd J, Ghukasyan V, Zylka MJ, Polleux F: **The F-BAR domains from srGAP1, srGAP2 and srGAP3 regulate membrane deformation differently**. *J Cell Sci* 2012, **125**:3390-3401.

34. •• Charrier C, Joshi K, Coutinho-Budd J, Kim JE, Lambert N, de Marchena J, Jin WL, Vanderhaeghen P, Ghosh A, Sassa T *et al.*: **Inhibition of SRGAP2 function by its human-specific paralogs induces neoteny during spine maturation**. *Cell* 2012, **149**:923-935.

This is an important work, which demonstrates that the gene encoding a downstream component of the Slit/Robo signaling pathway, srGAP2 has been subjected to a duplication step unique to humans during evolution. This generated truncated srGAP2 isoforms which antagonize the function of the ancestral srGAP2. The forced expression of the human truncated srGPA2 in mouse cortical neurons is sufficient to increase the density of cortical spines as found in human neurons.

35. Dennis MY, Nuttle X, Sudmant PH, Antonacci F, Graves TA, Nefedov M, Rosenfeld JA, Sajjadian S, Malig M, Kotkiewicz H *et al.*: **Evolution of human-specific neural SRGAP2 genes by incomplete segmental duplication**. *Cell* 2012, **149**:912-922.

36. Endris V, Wogatzky B, Leimer U, Bartsch D, Zatyka M, Latif F, Maher ER, Tariverdian G, Kirsch S, Karch D *et al.*: **The novel Rho-GTPase activating gene MEGAP/srGAP3 has a putative role in severe mental retardation**. *Proc Natl Acad Sci U S A* 2002, **99**:11754-11759.

37. • Carlson BR, Lloyd KE, Kruszewski A, Kim IH, Rodriguiz RM, Heindel C, Faytell M, Dudek SM, Wetsel WC, Soderling SH: **WRP/srGAP3 facilitates the initiation of spine development by an inverse F-BAR domain, and its loss impairs long-term memory**. *J Neurosci* 2011, **31**:2447-2460.

This study shows one of the first neuropsychological implications of a Slit–Robo pathway downstream component, srGAP3, and provides a cellular model in showing the detrimental effect of srGAP3 knockout on dendritic morphology.

38. Andrews W, Barber M, Hernadez-Miranda LR, Xian J, Rakic S, Sundaresan V, Rabbitts TH, Pannell R, Rabbitts P, Thompson H *et al.*: **The role of Slit–Robo signaling in the generation, migration and morphological differentiation of cortical interneurons**. *Dev Biol* 2008, **313**:648-658.

39. • Borrell V, Cardenas A, Ciceri G, Galceran J, Flames N, Pla R, Nobrega-Pereira S, Garcia-Frigola C, Peregrin S, Zhao Z *et al.*: **Slit/Robo signaling modulates the proliferation of central nervous system progenitors**. *Neuron* 2012, **76**:338-352.

The first evidence supporting a role for Slit/Robo in neurogenesis in the mammalian cortex. Robo is shown to act as a positive transcription activator of Hes1, a bHLH acting in the Notch pathway.

40. Sasaki T, Komatsu Y, Watakabe A, Sawada K, Yamamori T: **Prefrontal-enriched SLIT1 expression in Old World monkey cortex established during the postnatal development**. *Cereb Cortex* 2010, **20**:2496-2510.

41. Ip BK, Bayatti N, Howard NJ, Lindsay S, Clowry GJ: **The corticofugal neuron-associated genes ROBO1, SRGAP1, and CTIP2 exhibit an anterior to posterior gradient of expression in early fetal human neocortex development**. *Cereb Cortex* 2011, **21**:1395-1407.

42. Hannula-Jouppi K, Kaminen-Ahola N, Taipale M, Eklund R, Nopola-Hemmi J, Kaariainen H, Kere J: **The axon guidance receptor gene ROBO1 is a candidate gene for developmental dyslexia**. *PLoS Genet* 2005, **1**:e50.

43. Suda S, Iwata K, Shimmura C, Kameno Y, Anitha A, Thanseem I, Nakamura K, Matsuzaki H, Tsuchiya KJ, Sugihara G *et al.*: **Decreased expression of axon-guidance receptors in the anterior cingulate cortex in autism**. *Mol Autism* 2011, **2**:14.

44. Hu VW, Frank BC, Heine S, Lee NH, Quackenbush J: **Gene expression profiling of lymphoblastoid cell lines from monozygotic twins discordant in severity of autism reveals differential regulation of neurologically relevant genes**. *BMC Genomics* 2006, **7**:118.

45. Anitha A, Nakamura K, Yamada K, Suda S, Thanseem I, Tsujii M, Iwayama Y, Hattori E, Toyota T, Miyachi T *et al.*: **Genetic analyses of Roundabout (ROBO) axon guidance receptors in autism**. *Am J Med Genet B: Neuropsychiatr Genet* 2008, **147B**:1019-1027 http://dx.doi.org/10.1002/ajmg.b.30697.

46. • Bai G, Chivatakarn O, Bonanomi D, Lettieri K, Franco L, Xia C, Stein E, Ma L, Lewcock JW, Pfaff SL: **Presenilin-dependent receptor processing is required for axon guidance**. *Cell* 2011, **144**:106-118.

The identification of new silencing mechanism of Slit/Robo function in motorneuron involving presenilin-1 and the Netrin-1 receptor deleted in colorectal cancer.

47. Leyva-Díaz E, Del Toro D, Menal MJ, Cambray S, Susín R, Tessier-Lavigne M, Klein R, Egea J, López-Bendito G: **FLRT3 Is a Robo1-interacting protein that determines Netrin-1 attraction in developing axons**. *Curr Biol* 2014, **24**:494-508.